

P136**Opioid concept in human articular cartilage and chondrocytes: expression and possible effect in cartilage-damaging states**

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Purpose: Opiates have been of interest in research for their potential influence in stress, during injury, in surgery and during inflammation and infection. Both opiates and opioid-binding sites have been localized on cartilage in animal models, and the presence of opiates has been confirmed in human synovial membranes and fluid. The present work focuses on potential influence from such substances on human cartilage and chondrocytes.

Methods and Materials: In the present study a search for opioid receptors and opiate production was done on cartilage and monolayer cultures of chondrocytes obtained from patients undergoing total knee arthroplasty. We also studied the influence of opiates on important mediators of inflammation like TNF-alpha and IL-1 beta produced by cultured chondrocytes. Various methods such as PCR, immunohistochemistry, immunocytochemistry and qualitative Western-blot were used. To quantify beta-endorphin's effect on CREB and MAPK phosphorylation and TNF-alpha and IL-1 beta levels, we used semi-quantitative Western-blot and ELISA determination.

Results: We found that human chondrocytes do have mu-opioid receptors and can therefore be influenced by opiates. In addition, opioid stimulation of chondrocytes may regulate CREB, MAPK, IL-1 beta and TNF-alpha depending on the concentration of opiates and duration of incubation. Furthermore, the opioid effect on IL-1 beta was modulated via MAPK regulation. We did not find production of opiates in cartilage or chondrocytes.

Conclusions: Human articular chondrocytes may be influenced by opiates of both endogenous and exogenous origin via mu-opioid receptors. Opiates apparently can influence important processes such as inflammatory signals in human articular cartilage via CREB, MAPK and cytokine regulation.

P137**Expression of laminin binding receptors is a unique feature of immature nucleus pulposus cells**

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Purpose: Cells of the immature nucleus pulposus may have potential to promote matrix synthesis and stem cell differentiation for applications in intervertebral disc (IVD) matrix regeneration. We have previously identified expression of specific laminin receptors in the nucleus pulposus, but not anulus fibrosus regions of the immature rat and porcine intervertebral disc (e.g., integrin $\alpha 6$ and CD239). The objective of this study was to evaluate expression of relevant laminin binding proteins in human immature disc cells towards the goal of finding unique markers for the immature nucleus pulposus.

Methods and Materials: Lumbar IVDs of juvenile patients without evidence of degeneration (2-15 yo) were dissected for cryosectioning. Cells were also isolated from anulus fibrosus and nucleus pulposus regions of the IVDs and cultured for 2-4 days. After fixation, both sections and cells were blocked and immunostained with antibodies to human integrins $\alpha 3$, $\alpha 6$, $\beta 1$, $\beta 4$ (BD), a laminin-related tetraspanin (CD151, Santa Cruz) and Lutheran blood glycoprotein (CD239, Serotec).

Results: Immature nucleus pulposus tissue stained intensely positive for integrins $\alpha 3$, $\alpha 6$, $\beta 4$, CD239 and CD151 in a pattern that appeared as a dense network connected to cells. In contrast, no staining ($\alpha 3$, $\beta 4$, CD239) and very faint staining ($\alpha 6$, CD151) was found in the anulus fibrosus regions. Distinct differences were also noted between nucleus and anulus cells cultured in vitro.

Conclusions: Findings for differential expression of laminin receptors and related proteins in nucleus pulposus of immature IVD suggest that these proteins may be useful to distinguish immature cells and to phenotype regenerated matrix of nucleus pulposus.

P138**Comparison of invasive and non-invasive rigid body fixation for cartilage defect mapping using computer assisted surgery during knee arthroscopy**

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Purpose: In all indication guidelines for the different cartilage repair techniques in the knee the size of the cartilage defect is crucial. For the accurate determination of the defect size computer assisted surgery (CAS) may be a possible tool for the future. The implementation of CAS during arthroscopy of the knee should not cause further invasive procedures. The objective of the study was to compare the accuracy of invasive and non-invasive rigid body's fixation in the measurement of a cartilage defect during arthroscopy of the knee using computer software.

Methods and Materials: The study was performed on 2 cadaver knees were cartilage defects in different size and location on both femoral condyles were created. Afterwards the defects were assessed using a computer navigation system (OrthoPilot[®]) and special computer software (Cartilage Defect mapping: CDM[®]). The measurement included the circumference, maximal height and maximal width and the area of the defect. All measurement were done with invasive fixation of the rigid bodies in the femur and/or tibia and repeated with non-invasive fixation of the rigid bodies using rubber bands.

Results: There were no statistical significant differences in the calculated parameters for the cartilage defects using invasive compared to non-invasive rigid body fixation. All values outside the defined tolerance with more of 10% deviation were based on worst case scenarios like severe femur movements.

Conclusions: For further application of CAS during arthroscopy of the knee joint for cartilage defect mapping the use of non-invasive rigid body fixation can be recommended.

P139**Autologous osteochondral grafts in the treatment of focal chondral defects of the femoral head. An experimental study in rabbits.**

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Purpose: Comparison of the reconstructed rabbit femoral head articular surface microscopically and histologically

Methods and Materials: A 2,5 mm diameter, 3 mm depth iatrogenic osteochondral defect was created to the anterolateral weight bearing area of the right femoral head in 12 rabbits. 6 rabbits underwent autologous osteochondral transplantation. Donor site was the lateral condyle of the ipsilateral knee joint. The other 6 rabbits underwent subchondral drilling. Both groups were sacrificed after 6 weeks. The histological classification system of the ICRS was used. For statistical analysis we used the Mann - Wittney test

Results: According to the ICRS score, statistical significance was found for all variables between the 2 groups (subchondral drilling 6 weeks vs autologous osteochondral transplantation 6 weeks): articular surface ($p=0,0499$, matrix ($p=0,0039$, cell distribution ($p<0,00059$, subchondral bone ($p=0,0109$, cartilage mineralization ($p=0,09$ except cell population viability. Autologous osteochondral transplantation provided better results than subchondral drilling concerning: smoothness, continuity of the articular surface, dominance of the hyaline and mixed hyaline - fibrocartilage type of tissue, normality of subchondral bone and columnar distribution of cells. Viability of the cell populations was the same for both methods. Incorporation of the osseous part of the graft was successful in all cases without any necrosis of the femoral head.

Conclusions: Reconstruction of focal osteochondral defects of rabbit femoral heads through autologous osteochondral graft transplantation gives superior macroscopical and histological results in comparison to subchondral drilling